EPA/OPP MICROBIOLOGY LABORATORY
ESC, Ft. Meade, MD

Standard Operating Procedure
for
Glass Washing and Detergent Residues Test

SOP Number: SOP QC-03-03

Date Revised: 04-25-05

Prepared By: _____Date: ___/___/___

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Date Issued: ___/___/___

Withdrawn By: _____ Date: ___/___/___

Controlled Copy No.: _____

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1.0 SCOPE AND APPLICATION:

1.1 This protocol describes procedures for washing laboratory glassware and for shipping glassware to a contract laboratory for analysis. Detergents used in washing glassware may leave residues which are bacteriostatic. If residues are present, glassware may require additional rinsing to remove them (see refs. 15.1 and 15.2).

2.0 DEFINITIONS: None

3.0 HEALTH AND SAFETY: None

4.0 CAUTIONS:

4.1 The plates will be analyzed by a contract laboratory qualified to perform detergent residue testing (for example; QC Laboratories, Southhampton, PA). In preparation for shipment to the contract laboratory, the petri dishes must be placed in a cardboard box containing shipping peanuts or suitable packing material. Petri plates should be shipped within 36 hours after completion of sterilization.

5.0 INTERFERENCES:

5.1 All glassware will be inspected prior to use. Discard items with chips and etched surfaces.

6.0 PERSONNEL QUALIFICATIONS:

6.1 Personnel are required to be knowledgeable of the procedures in this SOP. Documentation of training and familiarization with this SOP can be found in the training file for each employee.

7.0 SPECIAL APPARATUS AND MATERIALS:

7.1 Miele Thermal Disinfector/Laboratory Glassware Washer Model G7783 serial number 16/18344823 located in room B206 (Glassware and Media Preparation Room) of the OPP Microbiology Laboratory at the ESC, Ft. Meade, MD.

7.2 Lancer1600 UP Laboratory Glassware Washer serial number 9G050714 located in room B206 (Glassware and Media Preparation Room) of the OPP Microbiology Laboratory at the ESC, Ft. Meade, MD.

7.3 Powder Detergent for Miele dishwasher

7.4 Liquid Detergent for Lancer dishwasher

7.5 Alconox Powdered Precision Cleaner

7.6 Glass Petri Dishes (20 X 100 mm)

7.7 Cardboard box containing shipping peanuts or suitable packing material

8.0 INSTRUMENT OR METHOD CALIBRATION: Not applicable

9.0 SAMPLE HANDLING AND STORAGE:

9.1 Detergents will be used and stored according to manufacturer's instructions.

9.2 Refer to section 4.0 for cautions associated with handling of petri dishes.

10.0 PROCEDURE AND ANALYSIS:

10.1 Glassware will be washed in the Miele Thermal Disinfector/Laboratory Glassware Washer and the Lancer1600 UP Laboratory Glassware Washer. Hand-washed items will be analyzed when hand washing is actively being utilized.

10.2 The Detergent Residues Test will be performed annually or when a new lot or different type of detergent is used. The test bacteria used is *Enterobacter aerogenes*.

10.3 The method includes three groups of petri dishes. Preparation numbers are assigned to each group of petri dishes according to SOP QC-15, Media Prep and Sterilization Run Numbers. One group (Group A) is washed and rinsed by the regular procedure used in the laboratory (for that dishwasher or by hand). Another group (Group B) is washed by the regular procedure used in the laboratory followed by additional rinses. There will be 6 additional "Final Rinse" cycles run manually in the Miele Washer and the Lancer Washer as well as for hand-washed items. Another group (Group C) is washed with the detergent at the dilution normally used in the laboratory but not rinsed.

10.3.1 In the Glass Washing and Detergent Residues Test, laboratory staff treat the petri dishes as described below and ship them to a vendor (for example, QC Laboratories) for analysis.

10.4 Machine Washed Glassware in the Miele Washer

10.4.1 Glassware will be washed in the Miele Thermal Disinfector/Laboratory Glassware Washer Model G7783 using the Universal wash program (Program E). This program includes a pre-wash and heated main wash (85°C), two tap water rinses and two DI water rinses, one unheated and one heated (70°C). All rinses use recycled rinse water from the washer reservoir. This will constitute the normal treatment that all machine-washed laboratory glassware receives in this dishwasher (group A).

10.4.2 Place the glass petri dishes (6 per group) in the dishwasher facing down and spaced evenly so the water will run out of the dish. Put half of the dishes in the lower compartment and half in the upper compartment.

10.4.3 Fill the detergent compartment with the amount suggested by the manufacturer of powder detergent and close the detergent cover. Record the amount of detergent used on the media prep sheet. Use the detergent as specified by the manufacturer; In addition, place one scoop of detergent directly on the washer door and a second one on the base of the dishwasher for the Pre-wash cycle.

10.4.4 Press the button for Program E “Universal Wash.”

10.4.5 Press the “Start” button. Allow the washer to run a complete cycle as would normally be run.

10.4.6 To achieve the additional six rinses for Group B, perform the following:

10.4.6.1 While pressing the T1 and T2 buttons, turn on the washer.

10.4.6.2 Select program “D.”

10.4.6.3 Continuously press the start button until “38" appears in the display. Note: As the display climbs from 1 to 38, you will hear various components in the machine cycle on and off. This is normal.

10.4.6.4 When “38" is reached, press T2 until 40°C rinse temperature is reached (default value = 70°C, 40°C is the minimum temperature allowed).

10.4.6.5 Washer will automatically stop at the end of the cycle (approximately 12-15 minutes).

10.4.6.6 Repeat the above steps until the petri dishes have been rinsed six times (a total of six additional rinses).

10.4.7 To achieve Group C, the washer must be stopped after the main wash cycle but before the first tap water rinse, approximately 20 minutes after starting the Universal cycle.

10.5 Machine Washed Glassware in the Lancer Dishwasher

10.5.1 Glassware will be washed in the Lancer1600 UP Laboratory Glassware Washer using Cycle 10, the designated standard laboratory wash program. This program includes a pre-wash and heated main wash (85°C), two tap water rinses and three DI water rinses, two unheated and one heated (70°C). All rinses use recycled rinse water from the washer reservoir. Liquid detergent is dispensed automatically through a metering pump at the rate of approximately 5 mL/sec in this dishwasher. The water consumption estimated per operation (according to the baskets used) is 30 L. The metering pump during normal wash is set to run for approximately 75 seconds to deliver the appropriate volume of detergent. This will constitute the normal treatment that all machine-washed laboratory glassware receives (group A) in this dishwasher.

10.5.2 Place the glass petri dishes (6 per group) in the dishwasher facing down and spaced evenly so the water will run out of the dish. Put half of the dishes in the lower compartment and half in the upper compartment.

10.5.3 Select cycle 10.

10.5.4 Press the “Start” button.

10.5.5 To achieve the additional six rinses for Group B, perform the following:

10.5.5.1 Run cycle 10 for the normal laboratory wash.

10.5.5.2 Run cycle 30 twice (cycle 30 is 3 cold demineralized rinses).

10.5.6 To achieve Group C, run cycle 20 (cycle 20 is the same as the normal laboratory wash with no rinse cycles).

10.6 Hand Washed Items. (When needed)

10.6.1 All hand washed items will be washed in Alconox detergent at the manufacturers' prescribed dilution (1%), rinsed with tap water, and then two times with DI water. This will constitute the normal wash treatment that all hand washed laboratory glassware receives. Wash petri dishes in this way to achieve the Group A treatment.

10.6.2 To achieve the additional six rinses for Group B, following the normal wash treatment, rinse the petri dishes six additional times with DI water (use DI water as it comes from the DI faucet not the DI water from the Barnstead unit).

10.6.3 To achieve Group C, wash the petri dishes as in 10.6.1 omitting the tap water or DI rinses.

10.7 Resource Management

10.7.1 Water Conservation. Laboratory personnel should be mindful of water consumption, and whenever possible, employ practices that minimize water use.

10.7.1.1 Specifically, laboratory personnel should run full loads whenever possible, and consider wash programs that conserve water and produce glassware or labware appropriate for its intended use.

10.8 Sterilization

10.8.1 Wrap each group of dishes in brown paper obtained from room A140 (Region 3), and record group designation and dishwasher type (A, B, or C) on outside of paper. Sterilize all plates for each Group (A, B, and C) in the usual manner (gravity cycle for 25

min). Record the sterilization run number on the appropriate Laboratory Detergent Residue Test Form (see 16.1 and 16.2)

10.9 Shipping

10.9.1 Once all plates have been sterilized, place the wrapped plates in a cardboard box with shipping peanuts or suitable packing material to prevent the plates from breaking during shipment.

10.10 Obtain a FedEx airbill from one of the Environmental Science Center's mailroom and complete it. Note the following items:

10.10.1 In section 1, enter laboratory's return address as Environmental Protection Agency, 701 Mapes Road, Ft. Meade, MD 20755-5350.

10.10.2 In section 2, enter "Laboratory Glassware" as the internal billing reference information.

10.10.3 In section 3, enter the contract laboratory's name, address, and phone number (for example, QC Laboratories' address as QC Laboratories, 1205 Industrial Blvd., Southampton PA 18966-0514 and the phone number as 215-355-3900).

10.10.4 In section 4a, check the "FedEx Express Saver" box

10.10.5 In section 5, check the "Other Pkg." box.

10.10.6 In section 6, do not check either of the "HOLD" or "WEEKEND" Delivery boxes. Also, check the "No" box under dangerous goods.

10.10.7 In section 7, check the "Sender" box and fill in the Fed Ex account number.

10.10.8 Obtain the "Sender's Copy" of the airbill from FedEx and place it in the Quality Assurance of Glass Washing and Detergent Residues Record Book.

10.11 Place the airbill inside the airbill holder. Peel off the paper from the backside (larger, lower section of paper) of the airbill holder to reveal an adhesive surface. Attach the airbill holder to the top of the shipping container. Do not peel off the narrow, top strip of paper from the airbill. FedEx must be able to pull out the airbill from the airbill holder.

10.12 Take the package to the Region III dock area for pickup by FedEx. The "Sender's Copy" of the airbill is retained by the Environmental Science Center receptionist for tracking/logging purposes.

10.13 As an alternative to section 10.13, the analyst may take the package directly to a local FedEx office for mailing.

10.14 Notify contract laboratory that package is being shipped for Detergent Residue Analysis (for example, QC Laboratory phone number 215-355-3900).

11.0 DATA ANALYSIS/CALCULATIONS:

11.1 When the report of inhibitory residue is received from the contract laboratory, record the testing results legibly and in indelible ink under the "Calculations and Conclusions" column.

11.2 The percent difference in colony counts between Treatment Groups will be calculated. Differences in colony counts from Group B of more than 15% indicate inhibitory residues.

12.0 DATA MANAGEMENT/RECORDS MANAGEMENT:

12.1 The reports of inhibitory residue analysis and completed forms (section 16.0) must be placed in the Quality Assurance of Glass Washing and Detergent Residues Test Book.

12.1 Data will be recorded promptly, legibly, and in indelible ink on the forms. Completed forms are archived in notebooks kept in a secure file room adjacent to offices D217. Only authorized personnel have access to these files. Archived data are subject to OPP's official retention schedule contained in SOP ADM-03, Records and Archives.

13.0 QUALITY CONTROL:

13.1 The Detergent Residues Test will be performed at least once yearly or when a new lot or different type of detergent is used. The test bacteria used is *Enterobacter aerogenes*.

13.2 The OPP Microbiology Laboratory conforms to 40CFR Part 160, Good Laboratory Practices. Appropriate quality control measures are integrated into each SOP.

13.3 For quality control purposes, the required information is documented on the appropriate form (see 16.0).

14.0 NONCONFORMANCE AND CORRECTIVE ACTION:

14.1 Any deviation from the protocol will be documented. If the regular wash procedure (Group A plates) is found not to be adequate for removal of inhibitory detergent residues then the wash procedure will be adjusted and the detergent residue test repeated until the wash procedure has confirmed that all inhibitory residues have been removed.

15.0 REFERENCES:

15.1 Bordner, R.H., J..A. Winter and P.V. Scarpino. eds. 1978. Microbiological Methods for Monitoring the Environment, Water and Wastes. EPA-600/8-78-017, Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio.

15.2 Eaton, A.D., Clesceri, L.S., Greenberg, A.E. eds. 1995. Standard Methods for the Examination of Water and Wastewater, 19th Edition. American Public Health Association, American Water Works Association, Water Environment Federation.

16.0 FORMS AND DATA SHEETS:

16.1 Laboratory Detergent Residue Test Form for Machine Washed Items

16.2 Laboratory Detergent Residue Test Form for Hand Washed Items

Laboratory Detergent Residue Test Form for Machine Washed Items
OPP Microbiology Laboratory

Test Information			
Washer Name/Model		Date Test Performed	
Detergent Name		Organism	
Lot No.			
Detergent Control #			

Glassware Preparation			
	Group A*	Group B*	Group C*
Date Washed			
Date Sterilized			
Sterilization Run #			
Prep No.			

Calculations and Conclusions			
	A\B	C\B	D\B
% Difference †			
Comments			

* The treatments/groups are defined as follows, A is normal wash, B is normal wash followed by six additional DI rinses, C is washed but not rinsed, D is sterile disposable petri dishes used as controls and supplied by the contract laboratory.

† Differences in average counts on Groups A-D should be less than 15% if there are no toxic or inhibitory effects.

Laboratory Detergent Residue Test Form for Hand Washed Items
OPP Microbiology Laboratory

Test Information			
Washer Name/Model		Date Test Performed	
Detergent Name		Organism	
Lot No.			
Detergent Control #			

Glassware Preparation			
	Group A*	Group B*	Group C*
Date Washed			
Date Sterilized			
Sterilization Run #			
Prep No.			

Calculations and Conclusions			
	A\B	C\B	D\B
% Difference †			
Comments:			

* The treatments/groups are defined as follows, A is normal wash, B is normal wash followed by six additional DI rinses, C is washed but not rinsed, D is sterile disposable petri dishes used as controls and supplied by the contract Laboratory.

† Differences in average counts on Groups A-D should be less than 15% if there are no toxic or inhibitory effects.